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Identifying possible non-thermal effects of radio frequency energy on inactivating food microorganisms

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ABSTRACT

Radio frequency (RF) heating has been successfully used for inactivating microorganisms in agricultural and food products. Athermal (non-thermal) effects of RF energy on microorganisms have been frequently proposed in the literature, resulting in difficulties for developing effective thermal treatment protocols. The purpose of this study was to identify if the athermal inactivation of microorganisms existed during RF treatments. *Escherichia coli* and *Staphylococcus aureus* in apple juice and mashed potato were exposed to both RF and conventional thermal energies to compare their inactivation populations. A thermal death time (TDT) heating block system was used as conventional thermal energy source to simulate the same heating treatment conditions, involving heating temperature, heating rate and uniformity, of a RF treatment at a frequency of 27.12 MHz. Results showed that a similar and uniform temperature distribution in tested samples was achieved in both heating systems, so that the central sample temperature could be used as representative one for evaluating thermal inactivation of microorganisms. The survival patterns of two target microorganisms in two food samples were similar both for RF and heating block treatments since their absolute difference of survival populations was < 1 log CFU/ml. The statistical analysis indicated no significant difference ($P > 0.05$) in inactivating bacteria between the RF and the heating block treatments at each set of temperatures. The solid temperature and microbial inactivation data demonstrated that only thermal effect of RF energy at 27.12 MHz was observed on inactivating microorganisms in foods.

1. Introduction

Radio frequency (RF) treatments have been proposed as a novel dielectric heating technology for rapidly heating agricultural and food products based on electromagnetic waves ranging from 3 kHz to 300 MHz. It has been successfully used for inactivating food microorganisms, such as *Escherichia coli* (Geveke and Brunkhorst, 2004; Kim et al., 2012; Li et al., 2017a), *Salmonella* (Gao et al., 2011; Ha et al., 2013; Nelson et al., 2002), and *Listeria* (Al-Holy et al., 2004; Awuah et al., 2005). The pasteurization mechanism with dielectric heating, including RF and microwave energy, has been debated and athermal (non-thermal) effects of electromagnetic energy are often proposed in the literature (Banik et al., 2003; Jacob et al., 1995; Shazman et al., 2007; Soghomonyan et al., 2016; Velizarov et al., 1999; Wang and Wang, 2016). Thermal effects are mostly considered on inactivating food microorganism since the cell death is only related to the heat generated by frictional interaction between the polarized molecules and charged ions in a product in response to the RF and microwave fields (Awuah et al., 2005; Shazman et al., 2007). Athermal effects are also claimed in many reports since high inactivation rates of microorganisms are observed at a low sample temperature under the electric fields (Saadi et al., 2014; Velizarov et al., 1999).

Thermal effects of RF or microwave energy are generally considered to be the only cause of inactivating food microorganisms (Carroll and Lopez, 1969; Gandhi, 1987; Gedye, 1997; Geveke et al., 2002; Hamoud-Agha et al., 2013; Hamoud-Agha et al., 2014; Ingram and Page, 2010; Shazman et al., 2007). However, there still has been a controversial discussion regarding the existence of athermal effects of RF and microwave energy on microorganism inactivation (Banik et al., 2003; Latorre et al., 2012; Shamis et al., 2012). Early researches report that microorganisms are inactivated more in RF or microwave systems than in water or oil bath methods (Culkin and Fung, 1975; Jacob et al., 1995). But the results cannot be repeated due to lack of the detailed heating parameters. The death rates of *Escherichia coli* in microwave heating are also higher than those obtained in conventional heat environment at the same temperature (Banik et al., 2003). Similar results were published by Dreyfuss and Chipley (1980) using *Staphylococcus aureus*, Singh et al. (1994) using *Cyano-bacteruim Nostoc*, and

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Schlisselberg et al. (2013) using *Salmonella* and *Listeria monocytogenes*. However, the differences of dynamic heating process, such as heating rate and uniformity between the two heating treatments, are not considered, making thermal and athermal effects difficult to distinguish. Cvengros et al. (2004) developed a thermostated oil bath to perform a same time–temperature profile with microwave heating treatment, but only the surface temperature of the samples measured using infrared sensors. Thus, it's extremely difficult to make a solid conclusion on existing athermal effects in RF or microwave treatments due to the lack of common experimental conditions (Banik et al., 2003; Jacob et al., 1995; Shamis et al., 2012; Velizarov et al., 1999; Wang and Wang, 2016).

Heating treatment conditions, including setpoint temperature, heating rate, and heating uniformity, have an important effect on thermal inactivation rates of given food microorganisms. For example, the different sample temperature results in various thermal inactivation rates since *D*-values of *E. coli* in ground beef are 11.85 and 1.63 min at 55 and 60 °C, respectively (Juneja et al., 1997). Even at the same temperature of 57 °C, the *D*-value of *E. coli* in mashed potato at the heating rates of 0.1 and 0.5 °C/min is significantly higher than that at 1, 5, and 10 °C/min (Chung et al., 2007; Chung et al., 2008; Kou et al., 2016). Beside these, the major challenge of the RF treatment is heating uniformity (Huang et al., 2016b; Jiao et al., 2015), which is a key factor related to the observed athermal phenomena (Hamoud-Agha et al., 2013). The non-uniform experimental temperature distribution is a major obstacle to identify athermal effects of RF energy on inactivating food microorganisms.

There is a need for reliable data that should be obtained in well-controlled conventional heating systems to simulate the RF heating process with the acceptable heating uniformity. A unique experimental thermal death time (TDT) heating block system has been successfully developed in our lab with a conventional source of heating energy for simulating RF treatments (Kou et al., 2016). The heating rates, setpoint temperatures, and holding times of samples can be precisely controlled by Visual-Basic software with proportional-integral-derivative (PID) algorithms in this TDT heating block system (Kou et al., 2016). This block system holds the potential to study the sole thermal inactivation rates of microorganism under the same heating conditions recorded during the RF heating process.

Objective of this study were to: (1) analyze the sample temperature profiles and heating uniformity in both heating block and RF treatments, (2) use the heating block system to simulate the RF heating process, and (3) compare the inactivation results of two bacteria strains in two food samples between the conventional heating and RF treatments.

2. Materials and methods

2.1. Equipment

A parallel plate RF heating system (COMBI 6-S, Strayfield International Limited, Wokingham, UK) of 6 kW at a frequency of 27.12 MHz with a free-running oscillator was used in this study. The system included a RF generator and an applicator containing two parallel electrodes inside a large rectangular oven. More detailed descriptions of the system can be found in Wang et al. (2010). The RF power was adjusted by changing the electrodes gap, and the RF power and heating rate increased as decreasing gap. A small polypropylene cylindrical test cell with dimensions of ϕ3.0 cm × 1.5 cm was selected as the sample holder, and the center temperature of the sample was measured using a fiber-optic temperature sensor system (HQ-FTS-D120, Heqi Technologies Inc., Xian, China) with an accuracy of ± 0.5 °C (Fig. 1).

A computer-controlled heating block system was used as a conventional thermal energy device. The heating block system consisted of a heating unit with 6 cells, a data acquisition/control unit, and a computer (Fig. 2). The setpoint temperature, heating rate, and holding time of the system were controlled by the customized Visual Basic software and two PID controllers (I32, Omega Engineering, Inc., Stamford, CT, USA), and the central sample temperature in the cell was measured by type-T thermocouple sensors (TMQSS-020-6, Omega Engineering Ltd., Stamford, CT, USA) with an accuracy of ± 0.5 °C, which were pre-calibrated by the program to keep consistent with the data from the fiber-optic sensor system. Detailed descriptions of the heating block system can be found in Kou et al. (2016).

2.2. Bacteria strains and cultivation

Two bacteria strains, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), from the College of Food Science and Engineering, Northwest A&F University (Yangling, China), were selected for the study. The cell suspensions of these two microbial strains were obtained and handled according to the previous study described in Li et al. (2017b). The final cell population was adjusted to a level of 10^9 CFU/ml and refrigerated (3 ± 1 °C) for no > 3 d before inoculation.

2.3. Sample preparation

Apple juice and mashed potato were used to study the possible athermal effects of RF energy on inactivating microorganisms. Apple juice (Huiyuan 100% Apple Juice, Yangling, China), as a representative acid and liquid food, was purchased from a local supermarket and stored in a refrigerator (BD/BC-297KMQ, Midea Refrigeration Division, Hefei, China) at 3 ± 1 °C and used within the expiration date. Mashed potato is often used as a semi-solid model food, because of its simple preparation, consistent chemical composition, and relatively homogeneous structure (Bornhorst et al., 2017; Campañone and Zaritzky, 2005). Dry mashed potato flakes (Simplot Australia Ltd., Tasmania, Australia) were mixed with distilled water to formulate into 15.38% wet basis (w.b.) mashed potato.

Each of 2 ml *E. coli* and *S. aureus* suspensions was inoculated into 200 ± 5 ml apple juice or 80 ± 1 g mashed potato to form composite samples with an initial population of 10^7–10^8 CFU/ml (CFU/g) for testing. All samples were kept at 30 °C in a water bath (SC-15, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) to get the same initial temperature before pasteurization experiments.

2.4. RF treatments

For apple juice, five cylindrical test cells were placed vertically in the middle of the electrodes, as shown in Fig. 1(a). The test cell at the center position was filled with 5 ml of inoculated samples and others with 5 ml of non-inoculated samples. The electrode gap of 9 cm was chosen based on preliminary experiments to obtain a fast heating rate. The sample core temperature was monitored and recorded using the fiber-optic sensor during the RF heating. After the temperature reached 35, 40, 45, 50, 55, 60, and 65 °C, the inoculated test cells were taken out from the RF system and placed in a sealed plastic bag, then put into ice water (≈4 °C, for at least 2 min) immediately for cooling, and then ready for enumeration.

For mashed potato, 130 g samples were added into 150 ml glass beaker, and a single cylindrical test cell filled with 5 g inoculated mashed potato was placed at the center of the upper layer in the beaker, as shown in Fig. 1(b). The electrode gap of 9.5 cm was chosen based on no arcing and fast heating rates during the preliminary tests. The other experimental conditions were the same as those with the apple juice.

After RF heating treatments, the samples were mixed with 20 ml sterile physiological saline in sterile flasks. Then, the mixture was 10-fold serially diluted in 0.9 ml of sterile physiological saline, and 0.1 ml of the diluent was spread onto LB agar. Where low levels of surviving cells were anticipated, 0.1 ml of undiluted homogenate was spread-